

# Responses of early, mid and late season apple cultivars to postharvest application of 1-methylcyclopropene (1-MCP) under air and controlled atmosphere storage conditions

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## Abstract

The potential for commercial application of 1-methylcyclopropene (1-MCP) to maintain quality of 'McIntosh', 'Empire', 'Delicious' and 'Law Rome' apples under air and controlled atmosphere (CA) storage conditions was investigated. These cultivars represent early, mid and late season apples with ripening rates ranging from fast to slow. 1-MCP gas concentrations used were 0.5, 1 and 2  $\mu\text{l l}^{-1}$ , generated from measured amounts of Ethylbloc<sup>TM</sup> powder. Fruit of each cultivar were removed from storage at 6 week intervals during 30 weeks in air, or at 8 week intervals during 32 weeks in CA, and evaluated after 1 and 7 days at 20°C. Effects of 1-MCP were greater in CA than air storage. A dose response of internal ethylene concentrations and flesh firmness to 1-MCP was found in 'McIntosh' and 'Law Rome', but 'Delicious' and 'Empire' ripening was generally prevented by all 1-MCP concentrations. 1-MCP reduced superficial scald incidence, and accumulations of  $\alpha$ -farnesene and conjugated trienols during air storage. The results indicate that the efficacy of 1-MCP is affected by cultivar and storage conditions, and that successful commercial utilization of the chemical will require understanding of these relationships. © 2000 Elsevier Science B.V. All rights reserved.

**Keywords:** 1-Methylcyclopropene; Softening; Ethylene; Quality; Storage

## 1. Introduction

Synthetic cyclopropenes such as 1-methylcyclopropene (1-MCP) block ethylene receptors and prevent ethylene effects in plant tissues for ex-

tended periods (Sisler et al., 1996a,b; Sisler and Serek, 1997). These chemicals therefore provide a valuable tool to investigate ethylene metabolism and have the potential to extend the storage life of horticultural products. Studies on 1-MCP responses have included commodities such as flowers (Serek et al., 1995; Sisler et al., 1996a), apples, bananas, oranges, plums, strawberries and tomatoes (Sisler and Serek, 1997; Abdi et al., 1998;

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Golding et al., 1998; Fan et al., 1999a,b; Ku et al., 1999; Porat et al., 1999). Important factors governing the efficacy of 1-MCP treatment are: (1) its effects are concentration  $\times$  time dependent; (2) the concentration required to inhibit ripening varies with the type of fruit and the stage of ripening at the time of treatment; and (3) although 1-MCP binding to the ethylene receptor is essentially irreversible, inhibition of ethylene effects may be overcome by production of new receptors.

1-MCP has recently become available as a stable powder (Ethylbloc<sup>TM</sup>) from which the gaseous form can be released by addition of dilute base. Prospects for commercial development of the compound are promising, especially for climacteric fruit such as apple in which control of ethylene production is associated with enhanced storage capability. Other inhibitors of ethylene binding, diazocyclopentadiene (DACP) and 2,5-norbornadiene, reduce apple fruit internal ethylene concentrations (IEC) and softening (Blankenship and Sisler, 1989, 1993). Recently, Fan et al. (1999a) showed that 1-MCP inhibits ethylene production, respiration, softening and loss of titratable acidity in five apple cultivars.

The primary objective of the work reported here was to examine the potential of 1-MCP for control of ripening in fruit of several apple cultivars under air and controlled atmosphere (CA) storage conditions. Apple cultivars vary widely in ethylene production, early season cultivars generally having higher rates of production than later season cultivars (Chu, 1988; Watkins et al., 1989), and may vary in their responses to 1-MCP application. Although 1-MCP binding to the ethylene receptor sites is irreversible, it appears that new receptors can be formed during the climacteric (Yen et al., 1995; Sisler et al., 1996a). Therefore, high ethylene producing cultivars, especially those that have entered the climacteric at the time of commercial harvest, might show less responsiveness to 1-MCP. We also tested the hypothesis that 1-MCP should inhibit development of the physiological storage disorder, superficial scald. Production of ethylene, and that of  $\alpha$ -farnesene, a sesquiterpene thought to be involved in scald development as a result of its oxidation (Whitaker et al., 1997), appear to be closely associated

(Watkins et al. 1993, 1995; Du and Bramlage, 1994). Furthermore, treatment of 'Granny Smith' apples with DACP inhibited both ethylene production and scald development (Gong and Tian, 1998). While our study was under review, Fan et al. (1999b) reported that 1-MCP delayed the rise in  $\alpha$ -farnesene production and accumulation of its oxidation products.

## 2. Materials and methods

### 2.1. Fruit source

Fruit used in these experiments were harvested from mature 'McIntosh', 'Empire', 'Delicious' and 'Law Rome' trees growing at the Cornell University orchards at Ithaca and Lansing, NY, on September 18, September 30, October 1, and October 12, respectively. Harvest dates were during the commercial harvest for each cultivar (Blanpied and Silsby, 1992). Uniformly sized fruit of each cultivar were randomized to provide 12 experimental units of 250 fruit, which were placed in 163 l plastic containers.

### 2.2. 1-MCP application

1-MCP was applied on the day of harvest to three replicate units of fruit for each 1-MCP concentration. Portions of 1-MCP as a powder (Ethylbloc<sup>TM</sup>; 0.43% active ingredient by weight) were weighed into test tubes to provide final gas concentrations of 0.5, 1.0 and 2.0  $\mu\text{l l}^{-1}$ . A solution of KOH:NaOH (50:50) supplied by the manufacturer was diluted to 1% concentrations, and added to the appropriate test tubes. Tubes were shaken and placed in each container, and an airtight lid closed within 30 s. Lids to each container were also taped to ensure a tight seal. After 7 h at 20–25°C, the containers were vented. Control fruit were kept under identical conditions without 1-MCP treatment.

### 2.3. Fruit storage and sampling

Twenty fruit from each experimental unit were transferred to an evaluation room kept at 20°C,

and remaining fruit were cooled overnight at 0.5°C. Thereafter, the cooled fruit of all cultivars were stored in air at 0.5°C (approx. 65% RH), or under CA conditions at 0.5°C ('Delicious', 'Law Rome') or 2°C ('McIntosh', 'Empire'). Atmospheres were applied via a flow-through system ('Empire', 'Delicious') or semi-static system ('McIntosh', 'Law Rome'). For the flow through system, fruit of each replicate were placed into 19 l glass jars, which were then stoppered and connected to an atmosphere mixing system which delivered a humidified flow ( $200 \text{ ml min}^{-1}$ ) of 2% O<sub>2</sub> and 2% CO<sub>2</sub>, the balance being N<sub>2</sub>. Atmospheres were monitored daily by gas chromatography (Fisher Gas Partitioner, model 1200, Fisher Scientific, Springfield, NJ). For the semi-static system, fruit were kept in an 850 l chamber and an atmosphere of 2% O<sub>2</sub> and 5% CO<sub>2</sub>, (2% CO<sub>2</sub> for the first 4 weeks of storage) for 'McIntosh', or 2% CO<sub>2</sub>/2% O<sub>2</sub> for 'Law Rome', maintained using an Oxystat II CA system (David Bishop, England). Atmospheres were kept within 0.2% of the target concentration throughout the experiment. Twenty fruit per replicate were sampled every 6 weeks for 30 weeks (air storage), or every 8 weeks for 32 weeks (CA storage), and transferred to the evaluation room.

#### 2.4. Assessment of fruit quality

After 1 and 7 days poststorage at 20°C, ten fruit were used for assessment of ripening and quality. IECs were measured on 1 ml samples of internal gas from the core as described by Alwan and Watkins (1999), except that the gas chromatograph used was a Hewlett Packard 5890, series II (Wilmington, DE). Firmness was measured on opposite sides of each fruit using an EPT-1 pressure tester (Lake City Technical Products, Lake City, Canada) fitted with an 11.1 mm diameter Effigi tip. Fruit were then assessed for external and internal disorders. Soluble solids concentration (SSC) was measured on combined juice collected from the penetrometer probe with a refractometer (Atago PR-100, McCormick Fruit Tech., Yakima, WA). Titratable acidity (TA) was measured on juice extracted from opposite 1/8th segments of bulked fruit samples using an auto-titrator (Mettler DL12, Hightstown, NJ).

At each sampling time fruit were assessed for the presence of disorders. On fruit showing scald symptoms, scald severity was rated using a scale based on the percentage of the surface area affected, where 1 = 1–10%, 2 = 11–33%, 3 = 34–66%, and 4 = 67–100%.

#### 2.5. Scald development in relation to $\alpha$ -farnesene and its conjugated trienol oxidation products

In a separate experiment designed to investigate the influence of 1-MCP on synthesis of  $\alpha$ -farnesene and its oxidation to conjugated trienols (CTols) in relation to scald development, 'Law Rome' fruit were harvested on October 12, 1998, and six experimental units placed in plastic containers. Three containers of fruit were treated with  $2.0 \mu\text{l l}^{-1}$  1-MCP and the other three sealed but not treated as described above. The three replicates of control and MCP-treated fruit were then stored in air at 0.5°C. After 0, 4, 8, 16 and 24 weeks in storage, groups of 10 fruit from each replicate were peeled rapidly and the peel was immediately frozen in liquid N<sub>2</sub> and stored at  $-80^\circ\text{C}$ . Frozen tissue samples used for analysis of  $\alpha$ -farnesene and CTol levels were shipped overnight to Beltsville, MD, under dry ice. Groups of 100 fruit of each treatment were taken out of storage 5 days after the 24-week sampling and assessed for scald incidence and severity a week later.

Two peel samples (3 g fresh wt.) were analyzed for each of the three replicates of control and 1-MCP-treated fruit from each of the five storage intervals (0, 4, 8, 16, and 24 weeks). Frozen tissue samples were pulverized in liquid N<sub>2</sub> and transferred to 50 ml screw-cap culture tubes containing 10 ml of HPLC-grade hexane. The tubes were flushed with N<sub>2</sub>, sealed, and agitated at 5°C for 1.5 h. Extracts were vacuum filtered through glass fiber disks and restored to 10 ml total volume. Aliquots of the extracts (1.0 ml) were transferred to 2 ml vials and the hexane evaporated under a gentle stream of N<sub>2</sub> without heating. The residue was dissolved in 400  $\mu\text{l}$  of HPLC-grade methanol and filtered through a  $0.45 \mu\text{m}$  PTFE membrane prior to HPLC analysis. Samples (80  $\mu\text{l}$ ) were injected manually into a Waters 600MS HPLC

system fitted with a  $4.6 \times 250$  mm, Luna C<sub>18</sub> column (Phenomenex, Torrence, CA). The mobile phase was methanol/acetonitrile/water (90:7.5:2.5) pumped at  $0.8 \text{ ml min}^{-1}$ . Absorbance at 232 nm ( $\alpha$ -farnesene) and 269 nm (CTols) was monitored by a Waters 490 programmable wavelength detector and data were gathered and processed using the Waters Baseline 810 program in a 286 PC.  $\alpha$ -Farnesene gave a single peak that eluted at 11.9 min and CTols gave a prominent peak at 5.8 min with a small shoulder at 6.0 min. Calculation of  $\alpha$ -farnesene and CTol concentration was based on their respective published molar extinction coefficients as previously described (Whitaker et al., 1997).

In addition to the analyses of peel samples from air-stored control and  $2 \mu\text{l l}^{-1}$  1-MCP-treated fruit, a set of analyses was also performed on peel tissue from 'Law Rome' apples treated with 0, 0.5, 1, and  $2 \mu\text{l l}^{-1}$  1-MCP and stored under CA (2% O<sub>2</sub>/2% CO<sub>2</sub>) for 30 weeks plus 1 day in air at 20°C (see Section 2.3). The tissue from this set of fruit was excised, frozen, shipped, and extracted as described above for peel from the air-stored apples.

## 2.6. Statistical analyses

Data were subjected to ANOVA using the General Linear Model (Minitab, State College, PA). Ethylene data were transformed to logarithms prior to analysis. Pearson correlation coefficients and regressions were calculated to assess relationships between log IEC and firmness.

## 3. Results

### 3.1. Fruit maturity at harvest

Although fruit were harvested during normal harvest windows, climacteric IECs were found in all cultivars as indicated by the percentage of fruit with  $\text{IEC} > 1 \mu\text{l l}^{-1}$  (Table 1). The highest IEC and lowest firmness was measured in the 'McIntosh' cultivar.

### 3.2. Cultivar responses to 1-MCP

#### 3.2.1. 'McIntosh' — air storage

1-MCP reduced the IEC within 24 h of treatment from  $32 \mu\text{l l}^{-1}$  (Table 1) to an average of  $10 \mu\text{l l}^{-1}$ , compared with  $43 \mu\text{l l}^{-1}$  in the control fruit. However, while averages included a portion of fruit that were climacteric, the majority had an IEC of less than  $0.5 \mu\text{l l}^{-1}$ . Increasing 1-MCP concentrations resulted in delayed increases in, and lower IEC, after various storage intervals plus 1 or 7 days at 20°C (Fig. 1A, B). However, responses to treatment were affected both by storage period ( $P < 0.001$ ), with inconsistent differences between IECs of fruit treated with 1 and  $2 \mu\text{l l}^{-1}$  1-MCP when analyzed at 0, 6 and 24 weeks, and by days at 20°C ( $P = 0.003$ ), with no effect of days for control fruit.

The rate of fruit softening was also reduced by increasing 1-MCP concentration (Fig. 2A, B), but this was affected by weeks of storage and days at 20°C ( $P < 0.001$ ). Both control and 1-MCP-treated fruit softened overall during storage, with

Table 1

Log IEC and flesh firmness of 'McIntosh', 'Delicious', 'Empire' and 'Law Rome' at harvest<sup>b</sup>

Factor	'McIntosh'	'Empire'	'Delicious'	'Law Rome'
Log IEC ( $\mu\text{l l}^{-1}$ )	$1.38 \pm 0.11$ (47) <sup>a</sup>	$0.55 \pm 0.07$ (30)	$0.22 \pm 0.19$ (33)	$0.192 \pm 0.857$ (27)
Flesh firmness (N)	$69.3 \pm 0.5$	$77.7 \pm 0.5$	$80.1 \pm 1.8$	$92.2 \pm 0.3$

<sup>a</sup> Values in parentheses represent percentage of fruit with  $\text{IEC} > 1 \mu\text{l l}^{-1}$ .

<sup>b</sup> Means represent three 10-fruit samples  $\pm$  SD.

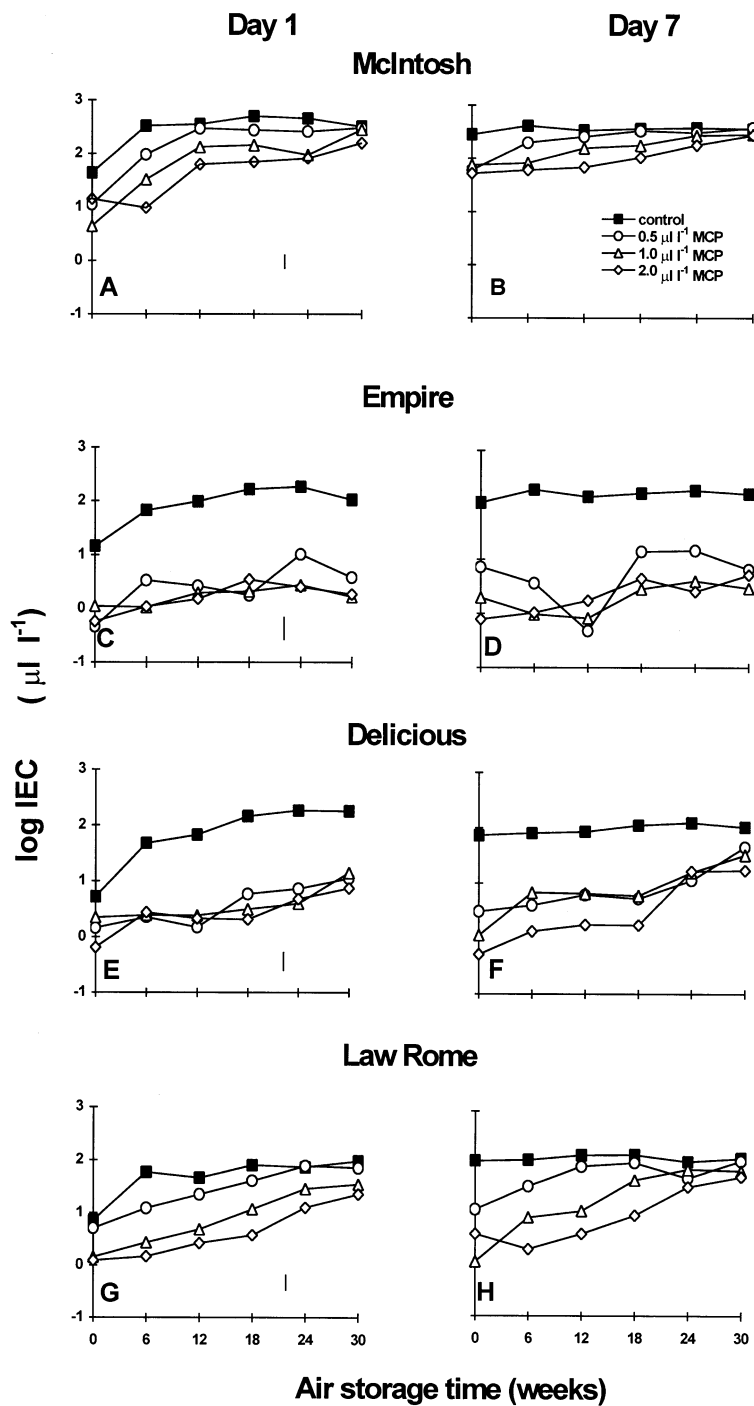


Fig. 1. Log IEC of 'McIntosh', 'Empire', 'Delicious', and 'Law Rome' apples treated with 0, 0.5, 1 and 2  $\mu\text{l l}^{-1}$  1-MCP and stored in air for up to 30 weeks. Fruit was removed from storage at 6 week intervals and assessed after 1 or 7 days at 20°C. Vertical bars represent LSD values at the 5% level for effects of treatment  $\times$  storage period  $\times$  post-storage ripening period.

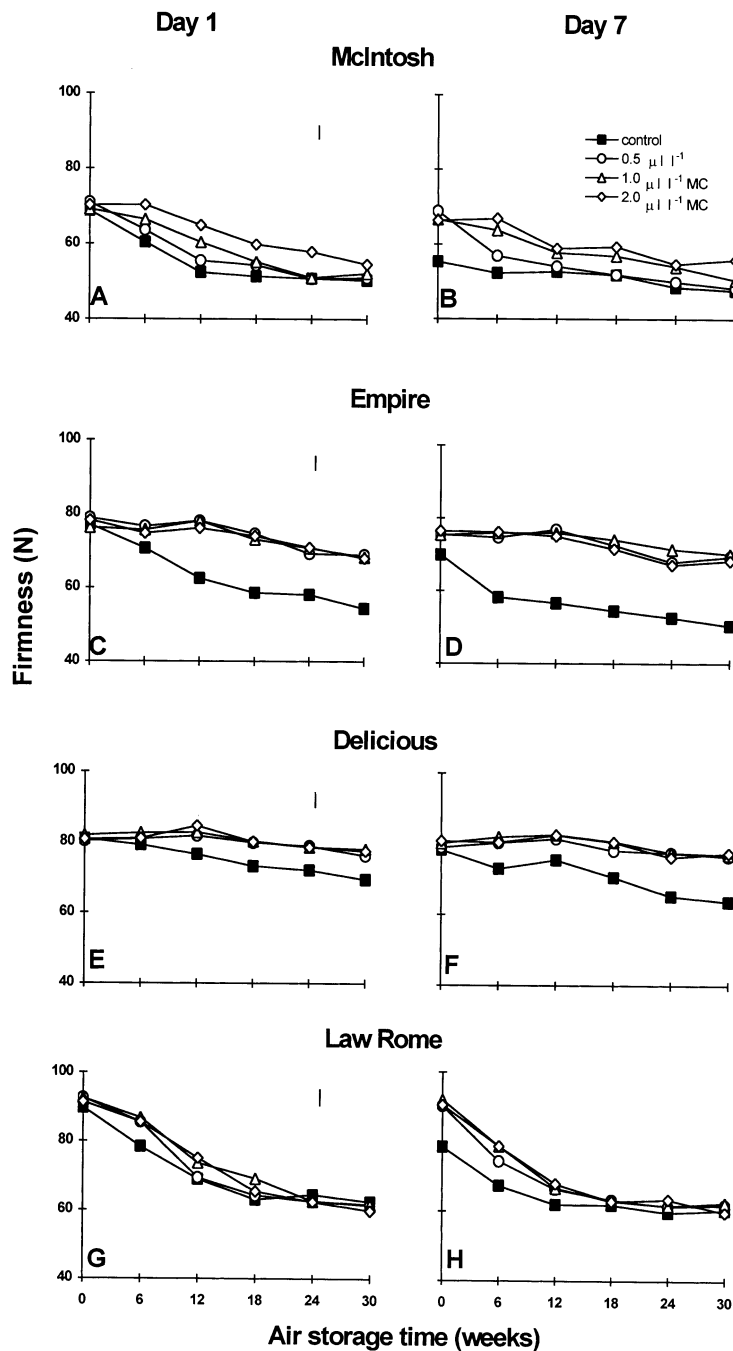


Fig. 2. Flesh firmness of 'McIntosh', 'Empire', 'Delicious', and 'Law Rome' apples treated with 0, 0.5, 1 and 2  $\mu\text{l l}^{-1}$  1-MCP and stored in air for up to 30 weeks. Fruit were removed from storage at 6 week intervals and assessed after 1 or 7 days at 20°C. Vertical bars represent LSD values at the 5% level for effects of treatment  $\times$  storage period  $\times$  post-storage ripening period.

relatively small differences between treatments. The variation of IEC and fruit firmness within samples is examined more closely in Section 3.3.

Fruit TA declined during storage from 0.52 to 0.23% but were higher overall in the 1-MCP-treated fruit than in control fruit ( $P < 0.001$ ); values were 0.34, 0.35, 0.37 and 0.37% for 0, 0.5, 1, and 2  $\mu\text{l l}^{-1}$  MCP, respectively. SSC was lower with higher 1-MCP concentration ( $P < 0.001$ ); values were 13.4, 13.3, 13.2 and 12.9% for 0, 0.5, 1, and 2  $\mu\text{l l}^{-1}$  1-MCP, respectively. Interactions between treatment, storage time and days at 20°C were significant ( $P < 0.001$ ) for both TA and SSC (data not shown).

Brown core increased in treated and untreated fruit during storage, but was consistently lowest in 1 and 2  $\mu\text{l l}^{-1}$  1-MCP-treated fruit (data not shown). By 24 weeks, incidence of the disorder was 93, 78, 53 and 43% in fruit from 0, 0.5, 1, and 2  $\mu\text{l l}^{-1}$  1-MCP treatments, respectively. Overall levels increased by 30 weeks, but were on average 21% lower in fruit from the two highest 1-MCP treatments.

### 3.2.2. 'McIntosh' — CA storage

After 1 day at 20°C, IEC of both control and 1-MCP-treated fruit from CA was much lower than those of fruit from air storage (Fig. 3A). During further ripening, IEC increased in all fruit, but was relatively lower in 1-MCP-treated than in control fruit until week 24 (Fig. 3B).

Fruit softening showed a similar, but inverse pattern to that of IEC on day 1, but overall 1-MCP treated fruit softened only 1.2 *N* compared with 8.6 *N* in control fruit during a further 6 days at 20°C (Fig. 4A, B).

Averaged TA was 0.41, 0.43, 0.42 and 0.39% for the 0, 0.5, 1, and 2  $\mu\text{l l}^{-1}$  1-MCP-treated apples, respectively, while SSC was lowered by 1-MCP, with values of 13.4, 13.3, 13.0 and 12.8% for 0, 0.5, 1, and 2  $\mu\text{l l}^{-1}$ , respectively. Treatment, storage and ripening interactions were evident for TA ( $P = 0.006$ ) and SSC ( $P < 0.001$ ), but no consistent trends were found (data not shown).

Incidence of disorders was negligible.

### 3.2.3. 'Empire' — air storage

IEC of control fruit increased during storage, and until week 18 increased further during the subse-

quent ripening period (Fig. 1E, F). IEC of 0.5  $\mu\text{l l}^{-1}$  1-MCP-treated fruit also increased, particularly at weeks 18 and 24.

Softening of control fruit was rapid over the entire storage period, while that of 1-MCP-treated fruit did not occur until after week 12 ( $P < 0.001$ ; Fig. 2E, F). During the poststorage ripening period, control fruit softened by 6.5 *N*, but 1-MCP-treated fruit by only 0.4 *N*.

TA averaged 0.27 and 0.34 units in control and 1-MCP-treated fruit, respectively, but was not affected by treatment until week 12 in either 1 or 7 day samples ( $P < 0.001$ ; data not shown). SSC averaged 14.3% in control fruit and 14.8% in 1-MCP-treated fruit ( $P < 0.001$ ). No interactions between treatment and any other factor were detected.

By 30 weeks, senescent breakdown was found in fruit from all treatments, being 23, 3, 6 and 6% in 0, 0.5, 1 and 2  $\mu\text{l l}^{-1}$  1-MCP-treated fruit, respectively.

### 3.2.4. 'Empire' — CA storage

IEC was generally low in all fruit at day 1 after removal from CA, but increased rapidly in control fruit over the next 6 days (Fig. 3E, F). Overall, IEC was 58.3, 1.3, 2.3 and 0.5  $\mu\text{l l}^{-1}$  in 0, 0.5, 1 and 2  $\mu\text{l l}^{-1}$  1-MCP-treated fruit ( $P < 0.001$ ), respectively. Increases in IEC over the 6 days at 20°C were low in the 1-MCP treated fruit, but were greater in the 1  $\mu\text{l l}^{-1}$  1-MCP treatment than other 1-MCP treatments at some removals ( $P < 0.001$ ).

Fruit softened little in CA storage, even those of the control except at 30 weeks (Fig. 4E, F). On average, 1-MCP-treated fruit were firmer (77.2, 76.9 and 77.9 *N* in 0.5, 1 and 2  $\mu\text{l l}^{-1}$  1-MCP fruit, respectively) than control fruit (73.9 *N*). Control fruit softened by 3.0 *N* during the ripening period, whereas 0.5, 1 and 2  $\mu\text{l l}^{-1}$  1-MCP-treated fruit softened by 0.2, 1.2 and 0.5 *N*, respectively ( $P = 0.026$ ).

Overall, TA was higher in 1-MCP treated fruit (0.35%) than in control fruit (0.32%;  $P < 0.001$ ), but while all interactions were significant, no consistent trends were identified. SSC was not affected by any factor (data not shown).

Senescent breakdown averaged 43, 16, 7 and 0% in 0, 0.5, 1 and 2  $\mu\text{l l}^{-1}$  1-MCP fruit, respectively.

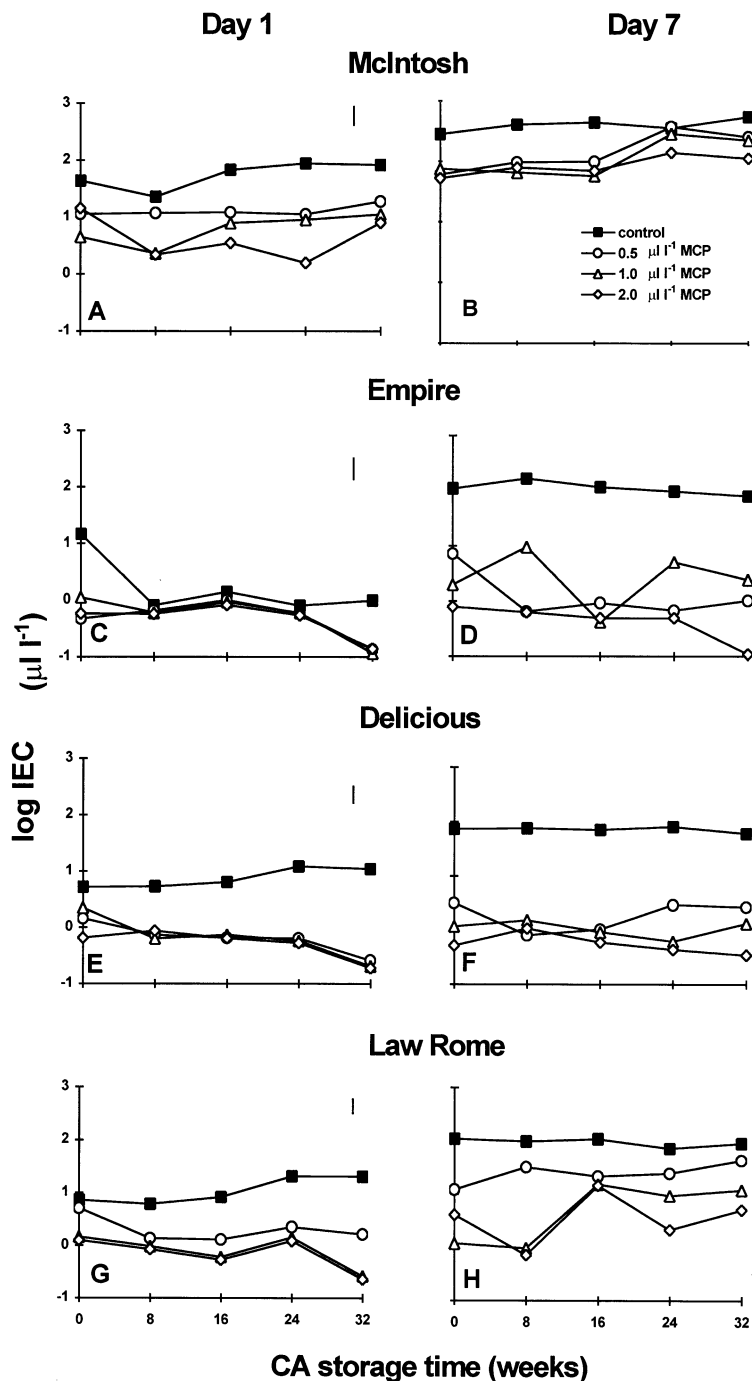


Fig. 3. Log IEC of 'McIntosh', 'Empire', 'Delicious', and 'Law Rome' apples treated with 0, 0.5, 1 and 2  $\mu\text{l l}^{-1}$  1-MCP and stored in controlled atmospheres for up to 32 weeks. Fruit were removed from storage at 8 week intervals and assessed after 1 or 7 days at 20°C. Vertical bars represent LSD values at the 5% level for effects of treatment  $\times$  storage period  $\times$  post-storage ripening period.



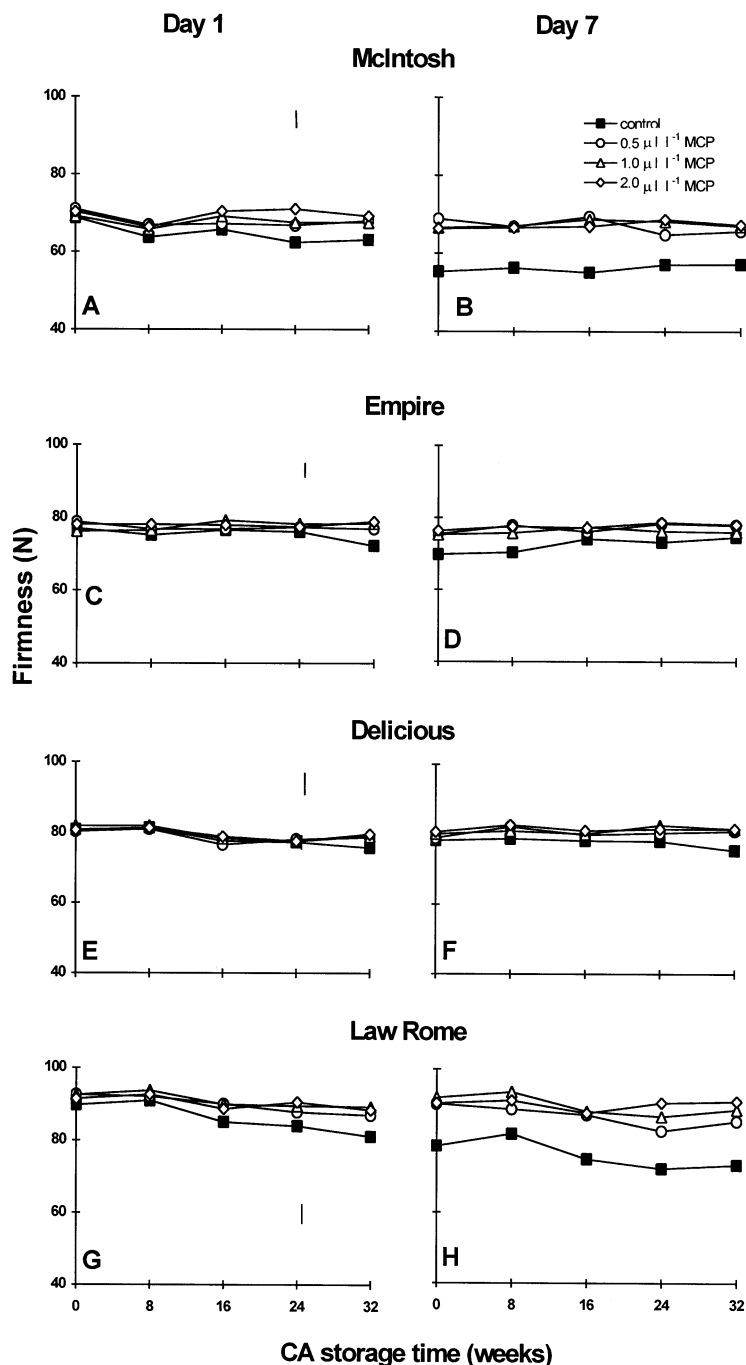


Fig. 4. Flesh firmness of 'McIntosh', 'Empire', 'Delicious', and 'Law Rome' apples treated with 0, 0.5, 1 and 2 µl l<sup>-1</sup> 1-MCP and stored in controlled atmospheres for up to 32 weeks. Fruit were removed from storage at 8 week intervals and assessed after 1 or 7 days at 20°C. Vertical bars represent LSD values at the 5% level for effects of treatment × storage period × post-storage ripening period.

### 3.2.5. 'Delicious' — air storage

IECs of control fruit increased greatly during storage and ripening averaging  $100 \mu\text{l l}^{-1}$ , but averaged only 8.7, 8.0, and  $4.9 \mu\text{l l}^{-1}$  in fruit from 0.5, 1, and  $2 \mu\text{l l}^{-1}$  1-MCP treatments, respectively (Fig. 1C, D). However, treatment effects interacted with storage period and days at  $20^\circ\text{C}$  ( $P = 0.001$ ), and by week 18, IEC began to increase during the poststorage ripening period.

1-MCP-treated fruit softened more slowly than control fruit both during storage and poststorage ripening (Fig. 2C, D). Treatment effects were greater with increasing storage period ( $P < 0.001$ ). Average loss of firmness was  $0.7 N$  in 1-MCP-treated fruit compared with  $4.1 N$  in control fruit between days 1 and 7 at  $20^\circ\text{C}$  ( $P = 0.003$ ).

Overall, TA was 0.11, 0.13, 0.14, and 0.13% in fruit treated with 0, 0.5, 1, and  $2 \mu\text{l l}^{-1}$  1-MCP, respectively. TA was not affected by treatment until week 12, but thereafter 1-MCP-treated fruit had higher acidity than control fruit on both days 1 and 7 ( $P = 0.001$ ). SSC was lower ( $P < 0.001$ ) in  $0.5 \mu\text{l l}^{-1}$  1-MCP fruit than in control fruit (14.1 and 14.6%, respectively), but highest in 1 and  $2 \mu\text{l l}^{-1}$  1-MCP fruit (14.9 and 14.8%, respectively).

Superficial scald only occurred in control fruit (53% at 30 weeks).

### 3.2.6. 'Delicious' — CA storage

Average IEC was 40, 1.4, 1.0, and  $0.6 \mu\text{l l}^{-1}$  in fruit treated with 0, 0.5, 1 and  $2 \mu\text{l l}^{-1}$  1-MCP, respectively (Fig. 3C, D). IEC of control fruit was greater after 1 day at  $20^\circ\text{C}$  as the storage period increased ( $P = 0.014$ ), and increased during 1 to 7 days of ripening at  $20^\circ\text{C}$  regardless of the duration of storage ( $P < 0.001$ ).

Firmness of control fruit averaged  $78.2 N$  compared with an average of  $80.2 N$  for 1-MCP-treated fruit ( $P < 0.001$ ). However, softening only occurred in control fruit ( $0.8 N$ ) over the 6 day ripening period (Fig. 4C, D).

TA was lower in 1-MCP-treated fruit than control fruit ( $P = 0.037$ ), but while control and  $0.5 \mu\text{l l}^{-1}$  1-MCP-treated fruit lost an average of 0.02% during 6 days at  $20^\circ\text{C}$ , no change occurred in fruit from the higher 1-MCP concentrations ( $P < 0.001$ ). SSC averaged 14.8, 14.2, 14.0 and 14.1% in 0, 0.5, 1, and  $2 \mu\text{l l}^{-1}$  1-MCP-treated fruit,

respectively ( $P < 0.001$ ). Storage time and days of ripening were also significant ( $P < 0.001$ ), but no interactions between factors were evident (data not shown).

No storage disorders were detected.

### 3.2.7. 'Law Rome' — air storage

Within 24 h of treatment, IEC of control fruit had increased to  $7 \mu\text{l l}^{-1}$ , while that of 0.5, 1 and  $2 \mu\text{l l}^{-1}$  1-MCP-treated fruit was 5, 1.4 and  $1.2 \mu\text{l l}^{-1}$ , respectively. The rise in ethylene was proportionally delayed and inhibited by higher 1-MCP concentrations during storage and poststorage ripening (Fig. 1G, H).

Fruit from all treatments softened during storage from an average of 89.7 to  $61.2 N$  ( $P < 0.001$ ). Although 1-MCP-treated fruit were on average  $3.8 N$  firmer than control fruit, they were not consistently firmer during storage and poststorage ripening ( $P = 0.05$ ; Fig. 2G, H).

TA averaged 0.29% in all MCP-treated fruit compared with 0.27% in control fruit ( $P < 0.001$ ), and was also affected by storage period and days at  $20^\circ\text{C}$ . However, no consistent treatment effects were detected (data not shown). SSC declined from 13.9 to 13.2% during storage and poststorage ripening ( $P < 0.001$ ), and was lower in 1-MCP-treated fruit ( $P = 0.038$ ). SSC was 13.6, 13.7, 13.4 and 13.3% in 0, 0.5, 1 and  $2 \mu\text{l l}^{-1}$  1-MCP-treated fruit, respectively.

Superficial scald developed during storage in control and  $0.5 \mu\text{l l}^{-1}$  1-MCP-treated fruit. By 18, 24 and 30 weeks, scald averaged 47 and 7%, 67 and 9%, and 90 and 44%, in the two treatments, respectively. At the last sampling, scald in the  $0.5 \mu\text{l l}^{-1}$  1-MCP-treated fruit was much less severe than in the control fruit. No scald was detected in fruit from the two highest 1-MCP concentrations.

### 3.2.8. 'Law Rome' — CA storage

IEC was affected by interactions between treatment, storage period and days at  $20^\circ\text{C}$  ( $P = 0.001$ ). IEC of control fruit was low, but increased with longer storage period (Fig. 3G). IEC in 1-MCP-treated fruit remained low overall, being 2.3, 0.9 and  $0.8 \mu\text{l l}^{-1}$  in 0.5, 1 and  $2 \mu\text{l l}^{-1}$  1-MCP-treated fruit, respectively. During the poststorage ripening period, IEC increased in fruit

Table 2

Pearson correlation coefficients between flesh firmness and log IEC for 'McIntosh', 'Delicious', 'Empire' and 'Law Rome' stored in air or CA for 30 and 32 weeks, respectively.

Cultivar	Air	CA
'McIntosh'	−0.820	−0.573
'Empire'	−0.818	−0.610
'Delicious'	−0.689	−0.344
'Law Rome'	−0.687	−0.706

from all treatments, but proportionately less with increasing 1-MCP concentration (Fig. 3H).

Firmness averaged 81.1 *N* in control fruit compared with 88.3, 90.4 and 90.2 *N* in 0.5, 1 and 2  $\mu\text{l l}^{-1}$  1-MCP-treated fruit, respectively ( $P < 0.001$ ), but was affected by interactions (Fig. 4G, H). Differences in firmness of 1-MCP-treated fruit were not consistent at each storage period ( $P = 0.02$ ), **but during poststorage ripening softened on average by 10, 3, 1 and 0 *N* in fruit from the 0, 0.5, 1 and 2  $\mu\text{l l}^{-1}$  1-MCP treatments, respectively ( $P < 0.001$ ).**

TA was not consistently affected by treatment (data not shown). However, the average SSC was 13.6, 14.3, 14.1 and 14.0% in fruit from the 0, 0.5,

1 and 2  $\mu\text{l l}^{-1}$  1-MCP treatments, respectively ( $P < 0.001$ ).

No storage disorders were detected.

### 3.3. Relationship between firmness and IEC

The relationship between firmness and IEC was examined for each cultivar and storage condition by correlation analysis. All correlations were significant ( $P < 0.001$ ) and were lower in CA-stored than in air-stored fruit for all cultivars except 'Law Rome' (Table 2).

In 'McIntosh' apples we further noted that IEC values were occasionally high at harvest (Table 1), ranging as high as 120  $\mu\text{l l}^{-1}$  in individual fruit. At this time, there was little variation in firmness. However, variation of both factors increased markedly at subsequent sampling times, including as early as 7 days after treatment at 20°C. At this time for example, the number of fruit that were unacceptably soft, using 55 *N* as an industry index, averaged 25% across 1-MCP treatments. The occurrence of soft fruit was associated with high IEC (Fig. 5). Although IEC increased in all fruit over time (Fig. 1A, B), the pattern of much

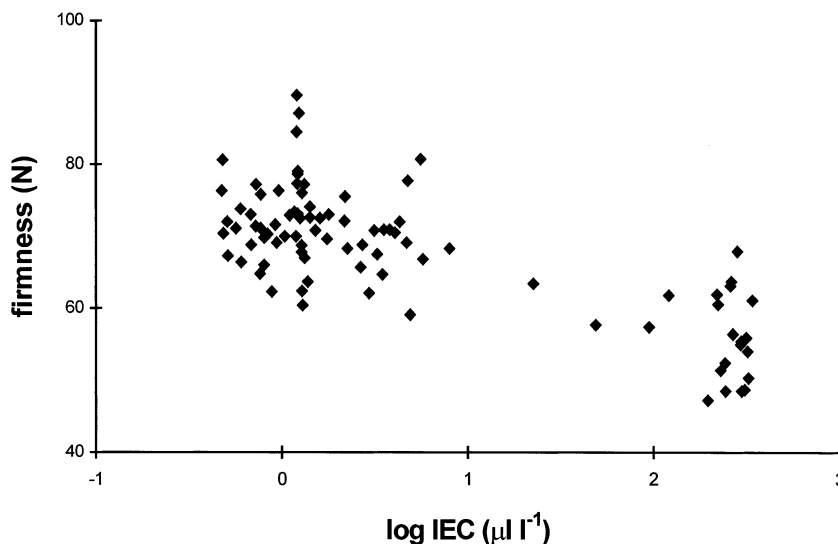


Fig. 5. Relationship between flesh firmness and log IEC of 'McIntosh' apples treated with 1-MCP (0.5, 1 and 2  $\mu\text{l l}^{-1}$  1-MCP, combined) after 7 days at 20°C. The  $R^2$  value for the regression relationship was 56.7%.

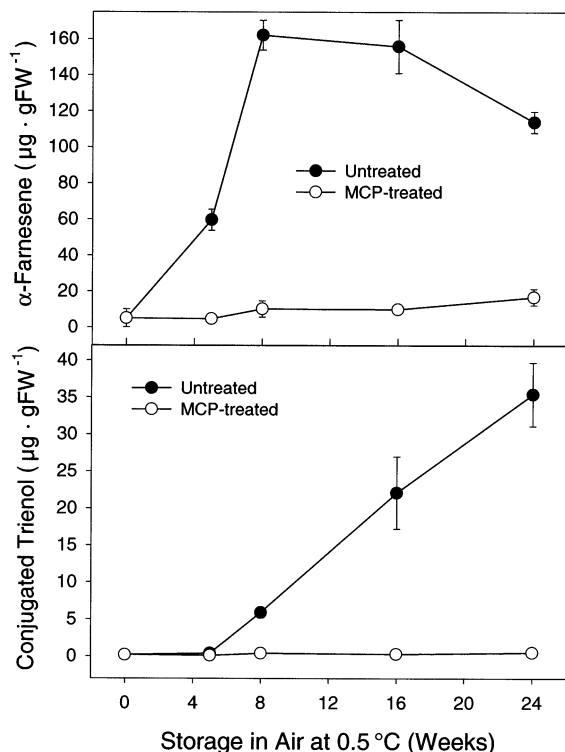


Fig. 6.  $\alpha$ -Farnesene and conjugated trienol content of apple peel from untreated control and  $2 \mu\text{l l}^{-1}$  1-MCP-treated 'Law Rome' apples over 0–24 weeks of storage at  $0.5^\circ\text{C}$  in air. Values represent the mean of two samples from each of the three replicates ( $n = 6$ ) and vertical bars indicate  $\pm$  SD.

higher IEC in individual fruit being associated with unacceptable softening was maintained. These data indicate that in the case of 'McIntosh', high IEC in fruit at harvest could not be controlled with 1-MCP at the concentrations used in this study.

### 3.4. Scald development in relation to $\alpha$ -farnesene and its conjugated trienol oxidation products

At harvest the level of  $\alpha$ -farnesene in peel tissue of 'Law Rome' fruit averaged just over  $5 \mu\text{g}$  per g fresh weight, but there was a large variation among the samples and three of the six analyzed had  $< 1 \mu\text{g g}^{-1}$ . Conjugated trienols averaged  $0.2 \mu\text{g g}^{-1}$  and were not detectable in samples with the least  $\alpha$ -farnesene. Over the first 4 weeks of  $0^\circ\text{C}$  storage in air,  $\alpha$ -farnesene increased nearly 12-fold and CTol doubled in peel of control fruit, but neither changed in peel of 1-MCP-treated fruit (Fig. 6). At 8 weeks the level of  $\alpha$ -farnesene in controls reached a maximum of about  $162 \mu\text{g g}^{-1}$  and CTol had begun to rise substantially. In contrast, levels of both compounds in treated fruit were only twofold greater than at harvest and about 15- to 16-fold lower than in the 8-week controls. Over 8–16 weeks, the  $\alpha$ -farnesene concentration in control fruit remained more or less constant, whereas CTol increased almost fourfold to about  $22 \mu\text{g g}^{-1}$ . No increase in either compound was noted in 1-MCP-treated fruit during this storage interval. By the final sampling at 24 weeks,  $\alpha$ -farnesene had begun to decline in peel of control fruit, while CTol had increased further to over  $35 \mu\text{g g}^{-1}$ . The level of  $\alpha$ -farnesene had increased in treated fruit, but only to about  $17 \mu\text{g g}^{-1}$ , with no significant increase CTol. At this time, scald incidence was 98% (severity score of 2.8) in the control fruit, and 1% (severity score of 1.0) in the 1-MCP-treated fruit.

Peel tissue samples were also analyzed at 30 weeks for fruit from the primary experiment to provide a comparison of air-stored and CA-stored

Table 3

Concentrations of  $\alpha$ -farnesene and conjugated trienols in peel tissue of 'Law Rome' apples stored 30 weeks at  $0.5^\circ\text{C}$  in air or CA conditions<sup>a</sup>

	Air-stored	CA-0 $\mu\text{l l}^{-1}$ MCP	CA-0.5 $\mu\text{l l}^{-1}$ MCP	CA-1 $\mu\text{l l}^{-1}$ MCP	CA-2 $\mu\text{l l}^{-1}$ MCP
$\alpha$ -Farnesene ( $\mu\text{g g}^{-1} \pm \text{SD}$ )	$80.0 \pm 4.8$	$93.4 \pm 4.4$	$54.5 \pm 19.0$	$44.6 \pm 14.8$	$30.3 \pm 8.7$
Conjugated trienol ( $\mu\text{g g}^{-1} \pm \text{SD}$ )	$33.2 \pm 3.8$	$8.2 \pm 1.4$	$3.2 \pm 1.3$	$2.6 \pm 1.1$	$1.6 \pm 0.6$

<sup>a</sup> CA-stored fruit were untreated or treated at harvest with a low, moderate or high level of 1-MCP (0.5, 1.0 and  $2.0 \mu\text{l l}^{-1}$ , respectively).

plus 0, 0.5, 1, or 2  $\mu\text{l l}^{-1}$  1-MCP-treated ‘Law Rome’ apples (Table 3).  $\alpha$ -Farnesene had continued to decline over the final weeks of air storage, and the concentration was actually somewhat lower than that in untreated, CA-stored fruit at this time. Nevertheless, the level of CTols remained high in the air controls and was fourfold greater than in the CA controls. Although the standard deviation among replicates was high for the 1-MCP-treated apples stored under CA, there was a clear trend toward decreasing concentrations of both  $\alpha$ -farnesene and CTols with increasing 1-MCP concentration. In the 2  $\mu\text{l l}^{-1}$  1-MCP-treated CA-stored fruit,  $\alpha$ -farnesene was 2.6-fold lower, and CTols were over 20-fold lower than in the air-stored controls. No scald was detected in CA-stored fruit from any treatment, however.

#### 4. Discussion

Fan et al. (1999a) found that 1  $\mu\text{l l}^{-1}$  1-MCP reduced softening in five apple cultivars stored in air for up to 6 months. Our study, which investigated four cultivars, with only ‘Delicious’ common between studies, indicated that the response of apple fruit to 1-MCP treatment at concentrations up to 2  $\mu\text{l l}^{-1}$  and subsequent storage in air can be cultivar dependent. In ‘McIntosh’ and ‘Law Rome’, the magnitude of IEC changes and softening were directly affected by 1-MCP concentration. In contrast, IEC of 1-MCP-treated ‘Empire’ and ‘Delicious’ increased little and fruit softened only gradually. Fruit types differ in 1-MCP concentrations that delay or prevent ripening (Sisler et al., 1996a; Sisler and Serek, 1997). Given the physiological variability among apple cultivars (Ye and Dilley, 1992), it is likely that optimum concentrations for 1-MCP treatment of air stored fruit will be greater for some cultivars than others.

Contrary to our original hypothesis, the effectiveness of 1-MCP was not related to harvest time of different cultivars. We assumed that earlier harvested cultivars, which generally have greater and more rapid increases in IEC than later harvested ones (Chu, 1988; Watkins et al., 1989),

might require higher 1-MCP concentrations to control ripening processes. This appeared to hold true for ‘McIntosh’, which under central New York conditions is often harvested after initiation of the climacteric (Blanpied and Silsby, 1992). The response of ‘Law Rome’, however, the cultivar with the lowest IEC in untreated fruit, also was directly affected by 1-MCP concentration.

CA storage generally resulted in markedly reduced IEC and reduced softening in 1-MCP-treated fruit, even in ‘McIntosh’ (presumably those fruit with low IEC at harvest) and ‘Law Rome’. The effectiveness of 1-MCP in reducing IEC during poststorage ripening of these cultivars, however, was related to treatment concentrations. The combination of 1-MCP treatment and CA storage appears to provide consistent control of softening for these cultivars. Interestingly, 1-MCP treatment was more effective in reducing softening than the rise in IEC in these fruit during poststorage ripening, suggesting that effects of ethylene on softening were at least partially separable. CA storage reduces ethylene production and action in apples (Gorny and Kader, 1997). Additive effects of low  $\text{O}_2$  and low ethylene in the storage environment on maintaining flesh firmness of apples have been shown, especially when daminozide has been used to delay IEC increases (Liu and Samelson, 1986; Graell and Recasens, 1992). In 1-MCP-treated fruit, however, negative correlations between IEC and firmness, though still highly significant, generally were lower in CA-stored than air-stored fruit.

Fan et al. (1999a) found that application of 1-MCP controlled ripening of climacteric as well as preclimacteric ‘Delicious’. Though we did not compare fruit of the two physiological stages in detail, all cultivars tested had a proportion of climacteric fruit at harvest. IEC in this subset of climacteric fruit was reduced by 1-MCP treatment within 24 h, except in ‘McIntosh’ and ‘Law Rome’ treated with the lowest 1-MCP concentration. ‘McIntosh’ had particularly high IEC in individual fruit at harvest. At each sampling time during storage, whether from air or CA, individual fruit from all 1-MCP treatments also had high IEC and were soft. The remaining fruit, which had lower IEC and were firmer, were presumably

those fruit that were treated while still preclimacteric. Therefore, even when data showed little change in firmness over time in air or CA, a proportion of the fruit was unacceptably soft from a marketing perspective. A rapid increase in ethylene binding sites during the climacteric in the tomato was shown by Yen et al. (1995), and although 1-MCP binding to the receptors is irreversible, inhibition of ethylene effects may be overcome by production of new receptors (Sisler et al., 1996a). The high rate of ethylene evolution in climacteric 'McIntosh' apples may result in an inability of 1-MCP to bind to sufficient sites to prevent ethylene action in these fruit. This effect may be analogous to that found in 1-MCP-treated bananas treated subsequently with propylene (Golding et al., 1998). Further studies are required to determine if higher 1-MCP concentrations than those tested here will reduce ethylene production of climacteric 'McIntosh' apples and maintain firmness.

The TA of 1-MCP-treated fruit was always higher than those of control fruit for all cultivars when stored in air, but in CA-stored fruit effects were inconsistent. Effects of 1-MCP on SSC were inconsistent in both air- and CA-stored fruit, and varied with cultivar. For example, 1-MCP-treated fruit in air had the lowest SSCs in 'McIntosh' and 'Law Rome', but the highest in 'Delicious' and 'Empire'. Fan et al. (1999a) found that TA was higher, and SSC higher or equal, in 1-MCP-treated compared with control fruit (only air-storage studied). In general, less ripe apples have higher TA, and lower SSC than more ripe apples, but the relationship between ethylene and these factors is not yet clear. Increases in SSC are presumably related to conversion of starch to sugars, but starch hydrolysis typically is initiated before the climacteric in apples (Brookfield et al., 1997). In tomato, metabolism of citrate, malate and starch, in contrast with polygalacturonase activity, appeared to be independent of ethylene (Jeffery et al., 1984). An interesting aspect of our data is that while the good correlation between maintenance of firmness and TA, commonly found in apples (Liu and Samelson, 1986; Fan et al., 1999a), was observed in air-stored fruit, this correlation was much weaker in 1-MCP-treated fruit stored under CA conditions.

Two types of storage disorder predominated in these experiments. The first type, in 'McIntosh' and 'Empire' was brown core and senescent breakdown. Brown core was predominantly the low temperature-induced type rather than senile brown core (Smock, 1977). How the former brown core would interact with effects on ethylene is not yet certain, but effects on senile brown core and senescent breakdown are fully consistent with 1-MCP's effect of delaying senescence. The second type of disorder that was greatly controlled by 1-MCP was superficial scald in 'Delicious' and 'Law Rome'. Fan et al. (1999b) have reported reduced incidences of superficial scald, soft scald, core flush, and greasiness in 1-MCP-treated fruit compared with control fruit.

We investigated the effects of 1-MCP on superficial scald in greater detail using 'Law Rome' apples. A long-standing hypothesis states that synthesis and oxidation of the sesquiterpene  $\alpha$ -farnesene are linked with development of superficial scald (Huelin and Coggiola, 1968). Although other factors are clearly important, such as changes in the antioxidant status of fruit tissues during storage (Anet, 1974; Rao et al., 1998), there is much evidence that accumulation of the conjugated triene oxidation products of  $\alpha$ -farnesene is involved in induction of scald (Whitaker et al., 1997, 1998). In addition, a number of studies have indicated that ethylene production influences  $\alpha$ -farnesene metabolism (Watkins et al., 1993, 1995; Du and Bramlage, 1994). Furthermore, three recent reports showed that inhibition of ethylene synthesis or action reduces production of  $\alpha$ -farnesene and CTols in apple peel during storage, and consequently prevents scald (Whitaker and Solomos, 1997; Gong and Tian, 1998; Fan et al., 1999b). In agreement with these findings, our results showed that blocking ethylene perception at harvest with  $2 \mu\text{l l}^{-1}$  1-MCP dramatically reduced the accumulation of  $\alpha$ -farnesene and CTols in peel of air-stored 'Law Rome' apples, and reduced the incidence of scald from near total to almost nil.

Because 1-MCP treatment delays or inhibits a number of senescence-related parameters in plant tissues, it could be argued that these effects were the basis of scald prevention, but the correlation

with marked inhibition of  $\alpha$ -farnesene synthesis and oxidation was very strong. CA storage alone was also prevented scald in 'Law Rome' apples, and our limited data indicates that CA conditions reduced synthesis and oxidation of  $\alpha$ -farnesene, although not as much as CA plus 1-MCP treatment. This is in accord with the report of Whitaker and Solomos (1997) that 1.5% O<sub>2</sub> flow-through CA prevented scald in fruit of 'Granny Smith' and markedly reduced accumulation of  $\alpha$ -farnesene and CTols in both 'Granny Smith' and 'Empire' apples.

In conclusion, these data show that 1-MCP is a postharvest chemical treatment that has tremendous potential for maintenance of apple fruit quality during storage and significant reduction of a major physiological storage disorder. The efficacy of 1-MCP is however, affected dramatically by cultivar and storage conditions, and successful commercial development will require a complete understanding of these relationships.

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